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Solvent-Based Elimination of Organic Matter from Marine-Collected Plastics

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Abstract: The physical-chemical characterization of plastic litter from the marine environment requires the prior removal of the biofouling attached to their surface without causing any degradation in the polymer. The absence of a standardized protocol for digesting biofouling and organic matter of both macro and microplastic samples extracted from seawater has been the main motivation for this research work, which aims to evaluate the effectiveness of different solvents (hydrogen peroxide, ethanol, a commercial enzymatic detergent, and potassium hydroxide) for the digestion of organic matter and biofouling in different samples recovered from the Spanish Atlantic and Mediterranean coast. Moreover, the potential effect of those solvents on the physical-chemical structure of polymers, four virgin plastic reference materials (low-density polyethylene, polyamide, poly(ethylene terephthalate) and polystyrene) without any type of prior degradation has been characterized in terms of Fourier transform infrared spectroscopy (FTIR) and optical microscopy. Results indicate that the hydrogen peroxide at 15% concentration applied for one week at 40 °C is the most effective solvent for organic matter and biofouling removal, without causing any apparent damage on the structure of plastic samples analyzed.

Keywords: marine litter; plastics; organic matter; digestion; solvents

1. Introduction

Marine plastic litter includes an increasing number of materials on which biofouling can develop. In addition, the production of new materials and combinations of materials boosts this trend. Biofouling or biological fouling is the non-desirable accumulation of deposits, especially living organisms, on artificial wetted surfaces. It can be classified into microfouling (biofilm and bacterial adhesion) and macrofouling (attachment of larger organisms like invertebrates and macroalgae) [1]. Thus, any surface in the marine environment will be colonized with biofilm formation, leading to biofouling [2]. However, the presence of the biofouling causes difficulties for the further identification and characterization of the sea-submerged materials, as in the case of plastics. As a result, for determining the degradation level of plastics from the marine environment according to different parameters (loss of mass of the polymer, fragmentation coefficient, degree of oxidation of the material, etc.) by gravimetry, FTIR, optical microscopy or tensile tests, it becomes necessary to clean their surface from any external material [3]. However, the retrieval of the biofouling may damage the surface of the material [4]. On the other hand, samples from oceanic waters contain large amounts of organic matter in the form of algae,

plankton and seeds, hence, detecting microplastics in filtered seawater samples requires a previous preparation of samples in order to digest the organic matter they may contain [5].

Thus, the high resistance of the biofouling and other organic matter in both types of marine samples makes it necessary to develop a sample preparation method with a high-capacity solvent that efficiently removes all the external organic material without degrading the plastic polymers, thus allowing their later identification and characterization [5].

Several authors have developed methods for the digestion of organic matter in marine samples by using solvents such as potassium hydroxide (KOH) [4,6–8], hydrogen peroxide (H_2O_2) [7,9–12], enzymatic methods [13,14] or ethanol (C_2H_5OH) [3]. Some of these protocols are developed for the extraction of microplastics from biological matrices (fish, mussels, crabs) [4,6,8]. KOH has been successfully used with a concentration of 10% for solving biological tissues at 40 °C, for 48 or 72 h, or at 60 °C for 24 h, while H_2O_2 showed a limited application as a digesting solution to extract microplastics from biological tissues at these conditions [4]. On the other side, H_2O_2 at 50 °C showed good performance solving biological materials within 96 h, but altered the color of PET and degraded PA and PS, according to Raman analysis.

Specifically, for digesting seawater samples for microplastics detection, Gago et al. (2018) [7] recommended to use KOH 10% for 72 h in a first step, and if necessary, an additional step using a 15% solution of H_2O_2 at 40 °C. Other authors suggested the use of a 30% H_2O_2 solution overnight to remove natural organic material from sediment samples [5]. The same 30% H_2O_2 solution is also used for microplastics detection in wastewater by Tagg et al. (2015) [11], demonstrating that up to 7 days of exposure at RT (room temperature) removed the majority of biogenic organic matter (opposite to Karami et al. (2017) [4] for digesting biogenic tissues) and had no significant impact on the FTIR spectra of polymers employed in the study, including PE, PS and PET. Nuelle et al. (2014) [10] also concluded that a 35% H_2O_2 solution had a good performance for biogenic organic matter removal in marine sediments after one week, but with no chemical characterization of polymers involved. Finally, Erni-Cassola et al. (2017) [12] showed that the adding of 30% H_2O_2 solution to filtered samples of seawater and beach sediments, kept at 60 °C for 1 h followed by a prolonged 7 h step at 100 °C, was effective for organic matter removal, even wood lignin and chitin, with no further chemical monitoring of polymers to check their potential degradation of materials.

Fenton's reagent, based on the use of H_2O_2 in the presence of a catalyst (Fe^{2+}), has been successfully applied for the removal of organic matter in other complex environmental substrates [15], such as soil and sludge [16], wastewater [17] or sediment samples of rivers [18]. The organic components and complexity of these matrixes could be very different from those of marine ones. However, according to authors' research (specific article in process), a good performance is shown by Fenton's reagent for the removal of organic matter from marine samples, but some materials like LDPE and PS may be altered during the process. This is probably due to the reaction temperatures, which can rise up to 89 °C in organic matrixes [16]. For that reason, Hurley et al. (2018) [16] recommend not to let reaction temperatures to exceed 40 °C to decrease the decomposition of hydrogen peroxide.

In addition, enzymatic digestion approaches have been successfully applied for the purification of biota-rich seawater samples and marine organisms with enzymes such as Proteinase-K [14] or technical grade enzymes (protease, cellulase, chitinase) [13], providing efficient purification for subsequent spectroscopic analysis, even if it constitutes a more expensive method. On the other hand, little information has been found on the specific cleaning of biofouling from sea-submerged materials. Napper and Thompson (2019) [3] used absolute ethanol prior to FTIR analysis, in order to remove any residue from marine PE samples, but providing no more details about the specific cleaning procedure. Other authors just used a paper tissue [19] or rinsed samples with deionized or distilled water to clean marine plastic materials [20,21], which seem to be insufficient solutions for a good performance of many further analyses such as FTIR. Regarding the use of acids and bases

as solvents (e.g., HCl, HNO$_3$ or NaOH), alterations were observed in plastic materials after some studies [4,6,8,16], as several plastic polymers (e.g., polyamide, polyoxymethylene, polycarbonate) react to strong acidic or alkaline solutions [5].

Thus, there seems not to be a standardized protocol for digesting biofouling and organic matter of both macro and microplastic samples from seawater, as revealed by some approaches being performed successfully in some studies but failing for others. The effectiveness of the solvent may depend on the origin or type of sample; as for example, if the organic matter to be removed is related to marine organisms or biogenic tissues, employed protocols may not be successful for those cases in which there is a greater predominance of vegetal matter. In addition, there is a lack of information on efficient methods for biofouling removal from submerged macroplastics, and furthermore, not all the studies reported chemical monitoring of plastics during the digestion treatment. However, the importance of guaranteeing polymer integrity cannot be over-stated when monitoring the degradation processes of plastics in the marine environment.

The aim of this work is, therefore, to establish an appropriate protocol useful for both: digesting the organic matter of seawater samples to allow microplastics identification, as well as digesting the biofouling for the surface treatment of submerged plastic materials prior to their characterization. Removing these organic matter and biofouling in a safe, timely and cost-effective way, while guaranteeing the integrity of materials, will allow the identification and characterization of both seawater macro- and microplastics, by minimizing interferences during later FTIR or other characterization analysis. For this purpose, we have tested the efficiency of H$_2$O$_2$, C$_2$H$_5$OH, a commercial enzymatic detergent and KOH on different marine samples, additionally verifying through optical microscopy and FTIR analysis their effect on the physical-chemical structure of four reference virgin plastic materials: low-density polyethylene (LDPE), polyamide (PA), poly(ethylene terephthalate) (PET) and polystyrene (PS), four of the most common polymers found in the marine environment [22,23].

2. Materials and Methods

Five solvents were evaluated: H$_2$O$_2$ (15% (v/v)), C$_2$H$_5$OH (96% (v/v)), a commercial enzymatic detergent (KH7 stain remover, KH Lloreda, S.A., Barcelona, Spain) and KOH (20% and 10% (v/v)).

The H$_2$O$_2$, purchased from Labkem at 30% (v/v), was diluted in distilled water to 15% (v/v). The 96% (v/v) extra pure Pharmpur® C$_2$H$_5$OH was supplied by Scharlau (Barcelona, Spain) In addition, a commercial product (KH7 stain remover) consisting of sodium lauryl ether sulphate, sodium dodecylbenzene sulfonate and ethoxylated fatty alcohol with less than 5% enzyme content was used as an enzymatic detergent at 100% concentration. KOH was purchased from Panreac Applichem as pellets with 85% purity and then diluted with distilled water to 20% and 10% (v/v) solutions, respectively.

Three types of samples were tested (aquarium samples (AQ), raw marine plastic samples (MS) and reference samples (RS)) and the effect of five mentioned solvents on them was analyzed. AQ samples corresponded to filtered subsurface seawater samples, containing organic matter to digest, while MS and RS samples corresponded to environmental and reference laboratory plastic samples, respectively.

AQ samples were collected from the inlet pumping of the Aquarium of Donostia-San Sebastián (north of Spain, Atlantic coast) on 8 May 2019, and were the result of filtering 2000 L of water from La Concha Bay at a depth of 4 m, through a primary 5 mm sieve and a secondary 100 μm sieve. The purpose of tests performed to aquarium samples (AQ) was to verify the digestion capacity of organic matter in the form of algae, zooplankton and biological matter present in seawater samples, which would allow the subsequent detection of potential microplastics.

The MS samples were raw marine plastic samples containing biofouling and impurities adhered on their surface. The test on MS samples were performed in order to check the capacity of solvents to remove the biofouling and impurities adhered on environ-

mental macroplastics. With this purpose, two different (white and transparent colored) LDPE macroplastic film sheets (20 × 10 cm) were collected at the surface waters in the Mediterranean coast (L'Ametlla de Mar, Catalonia) in August 2019.

The RS samples corresponded to four reference laboratory commercial plastic materials (PET, LDPE, PA and PS) without any initial damage due to aging, in order to ensure the detection of any potential harmful effects due exclusively to the digestion process in the tested polymers. FTIR spectra of the initial RS reference materials are shown in the FTIR analysis section, identified as LDPE RS0d, PA RS0d, PET RS0d and PS RS0d, verifying no initial damages in any of the four reference materials. These polymeric materials were selected as they correspond to four of the most commonly reported polymers at the marine environment [22,23]. In the test with RS, the effect of solvents on the physical-chemical structure of the four reference plastic materials was analyzed. As PET samples, recently purchased commercial bottles (Nestlé Aquarel) were selected, without recycled components, transparent, colorless and with an almost non-profiled surface to facilitate subsequent cleaning. LDPE samples came from a batch of commercial white film bags without any printing; PA ones from a new aquaculture lantern net, while PS was taken from the filling material of a typical commercial packaging.

In each test, the corresponding samples were placed into glass beakers: 30 mL for AQ samples, pieces of 2 cm^2 of each material for RS samples and two pieces of 25 cm^2 for MS samples. Then, the required solvent was added to each beaker until the materials were covered (40 mL for RS and AQ samples and 100 mL for MS samples), as shown in Figure 1a. All the laboratory material used was glass-made, to avoid any other plastic material contamination or reaction during the solvent-based cleaning processes which may alter the evaluated materials. Tests were performed at 40 °C (except for 10% (*v/v*) KOH, performed at RT, based on the work of Gago et al. (2018) [7], who successfully employed those conditions) over a period of one week, making visual observations of samples after 24 and 72 h and one week, and noting observed changes. Results of the visual inspection of organic matter and biofouling removal for AQ and MS marine samples were classified according to a 1–4 numeric scale, reflecting the degree of removal of organic matter and biofouling from the samples after the tests: '1' refers to no changes, '2' to a low elimination level, '3' to a high elimination level and '4' to the complete elimination of organic matter and biofouling (see Figure 1). To avoid any additional potential physical alterations to the samples, stirring or ultrasonic methods were not applied during the process. In the case of tests with MS, subsamples were cleaned after the test with distilled water and 96% (*v/v*) ethanol applied through a tissue paper, to observe the removal degree of initially present biofouling and impurities. Remaining AQ and MS samples were kept in the solvents during three more months at RT after the test.
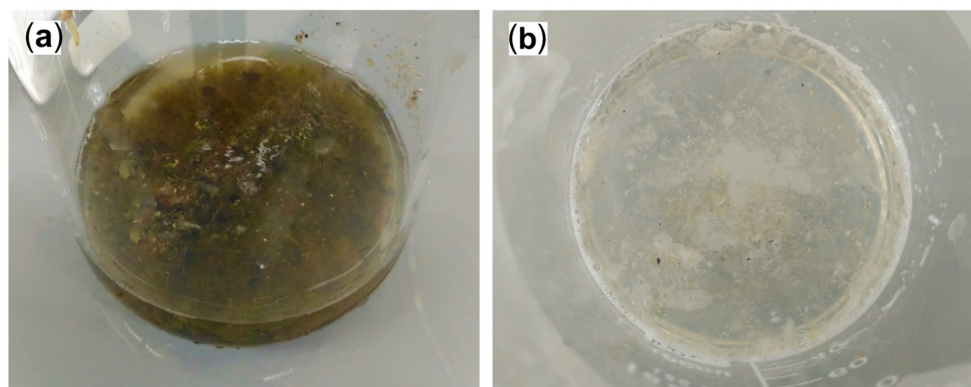
Figure 1. AQ sample, before the test (level 1 of the scale) (**a**) and after one week (**b**) of treatment with H$_2$O$_2$ at 15% (*v/v*) (level 4 of the scale).

With regard to RS samples, in addition to visual observation, subsamples of each material were collected for their physical-chemical characterization at the beginning, at 24 and 72 h, and one week after the beginning of the test. After the test, subsamples were cleaned with distilled water and ethanol, and dried at 50 °C for FTIR and optical microscopy characterization. In the case of LDPE and PS samples, both floating materials in the solvents, the analysis of two opposite sides of the samples at 72 h was included, to check for potential differences between the side in contact with the solvent and the side on the surface.

FTIR spectra were recorded using a Nicolet Nexus 670 infrared spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Golden Gate ATR system with ZnSe crystal, performing 32 scans with 4.0 resolution, at 4000–650 cm^{-1} range. Nikon 80i optical microscope (Nikon Instruments Inc., Melville, NY, USA) was used for surface observations of the materials after the tests, in 100× magnification, to detect potential physical changes.

3. Results and Discussion

3.1. Visual Observation

H_2O_2 showed the best performance for removing organic matter and algae fibers for AQ and MS samples (Table 1), as suggested by Gago et al. (2018) [7]. After 24 h, practically all the algae in the AQ samples were removed and after one week, all the organic matter disappeared (Figure 1b). Likewise, most of the impurities and biofouling present at MS samples also disappeared (Figure 2).

Table 1. Results of the visual inspection of organic matter and biofouling removal for the AQ and MS tests. Scale 1→4 refers to the degree of removal of organic matter and biofouling in the samples: 1: no changes, 2: little elimination, 3: high elimination, 4: total elimination. ('-' test not performed).

Test Parameters		Aquarium Samples(AQ)				Raw Marine Plastic Samples (MS)			
	Testing period (day)	1	3	7	97	1	3	7	97
	Test temperature (° C)	40	40	40	RT	40	40	40	RT
Solvents	H_2O_2 (15% *v/v*)	3	3	4	4	3	3	3	3
	Ethanol (96% *v/v*)	1	1	1	1	2	2	2	2
	Enzymatic detergent (100% *v/v*)	1	2	3	3	2	2	2–3	2–3
	KOH (20% *v/v*)	2	2	2	2	2	2	2	2
	KOH (10% *v/v*)	2	2	2	2	-	-	-	-
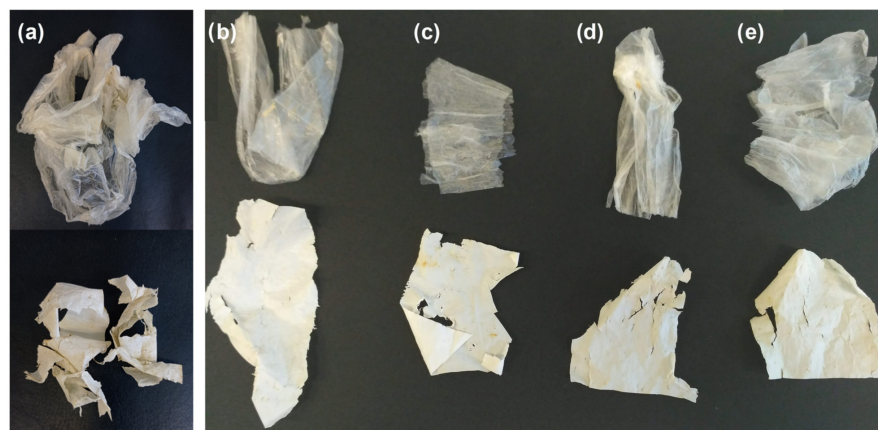
Figure 2. Initial MS samples (upper line corresponding to transparent LDPE sample, and lower line corresponding to white colored LDPE sample) (**a**) and MS samples tested with different solvents after one week: enzymatic detergent at 100% (**b**), H_2O_2 at 15% (**c**), KOH at 20% (**d**) and ethanol at 96% (**e**). After tests, samples were cleaned with distilled water, ethanol and a tissue paper.

KOH (at any of two concentrations tested) and ethanol resulted ineffective for solving organic matter and biofouling in AQ and MS samples, even after three months of tests. In contrast, the enzymatic detergent appeared to be moderately effective in solving the organic matter of AQ samples, as well as in removing the biofouling from MS ones, as concluded by Cole et al. (2014) [14], although their proteinase-K treatment resulted in a better performance for organic matter digestion than in our case.

Comparing to studies of other authors [4,6,8], KOH at the above mentioned concentrations may result for digesting biological tissues, but not for vegetal fibers such as algae or zooplankton as in this case. Additionally, Gago et al. (2018) [7] recommended a first step of treatment in KOH 10% to digest seawater samples, but according to our results, this step is not effective and can be suppressible. Observations after three months can be also found in Table 1 for AQ and MS samples at RT, with no significant changes observed.

Regarding reference RS samples, none of the four materials tested (LDPE, PA, PET and PS) showed visual evidence of affection by treatment with H_2O_2 and ethanol (images shown in Table 2) apart from the loss of PA dye evidenced after 24 h. PS took on a brownish color after 72 h, most likely due to the PA dye, as all four materials were placed in the same beaker for each solvent. There is neither visual evidence of affection on RS samples by the enzymatic detergent, as concluded by Cole et al. (2014) [14] for PS, PE and PA, apart from the significant loss of PA dye detected in RS samples from the beginning, resulting into a brown coloring of PS after one week. In the case of KOH treatment, the surface of PET from RS samples became translucent after 24 h with KOH at 20% and after 72 h with KOH at 10% (shown in Table 2). After one week, PET surface showed granules and became very fragile (as shown together with the FTIR analysis section), which would suggest a potential affection by the KOH. As for the other solvents, PA shows loss of dye after 24 h with KOH at 20%, and after one week with KOH at 10%.
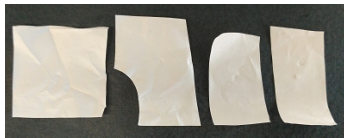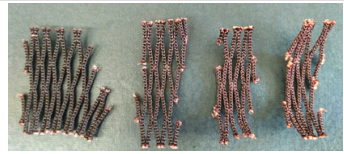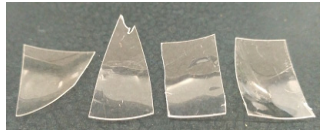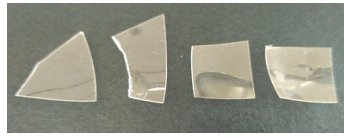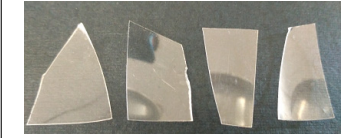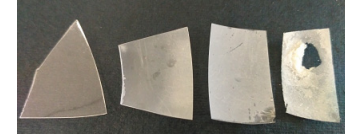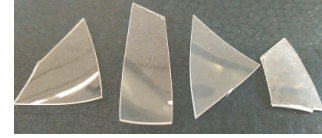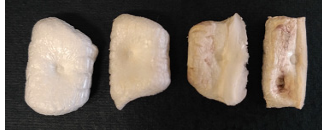
3.2. FTIR and Optical Microscopy Analysis of RS Samples

Given that H_2O_2 (15% v/v) and enzymatic detergent (100% v/v) provided the best performance on organic matter and biofouling removal from AQ and MS samples without any important visual effect on the RS plastic samples, we then analyzed on FTIR and optical microscopy for any potential chemical change in the materials. We also analyzed KOH-treated RS samples, as this solvent is widely used in the bibliography [4,6–8], despite that—according to our tests—some RS reference plastic samples showed visual evidence of affection.

3.2.1. Samples Tested with H_2O_2 at 15% (v/v)

No degradation effect is evident in the FTIR analyses, as very similar spectra are found for all analyzed polymers from RS samples throughout the test with H_2O_2 at 15% (Figure 3A). Likewise, no difference was found in the case of LDPE and PS between the sides in contact with the solvent and those that were not. Results are in accordance with Tagg et al.'s (2015) [11] results, who found no significant impact on the FTIR spectra of PE, PS and PET after 7 days of exposure to H_2O_2 at a higher concentration of 30%. Optical microscopy analysis also shows no damage evidence in the surface of materials due to H_2O_2 at 15% treatment after one week, as shown in Figure 3B.

Table 2. Visual observation of reference samples (RS) during the test with different solvents. Samples are identified as RSXdY, where X and Y indicate the number of days and the treatment temperature, respectively.

| Solvent	H_2O_2 (15% *v/v*)	Ethanol (96% *v/v*)	Enzymatic Detergent (100% *v/v*)	KOH (20% *v/v*)	KOH (10% *v/v*)
Period Material	RS0d/RS1d40/RS3d40/RS7d40	RS0d/RS1d40/RS3d40/RS7d40	RS0d/RS1d40/RS3d40/RS7d40	RS0d/RS1d40/RS3d40/RS7d40	RS0d/RS1dRT/RS3dRT/RS7dRT
LDPE					
PA					
PET					
PS					
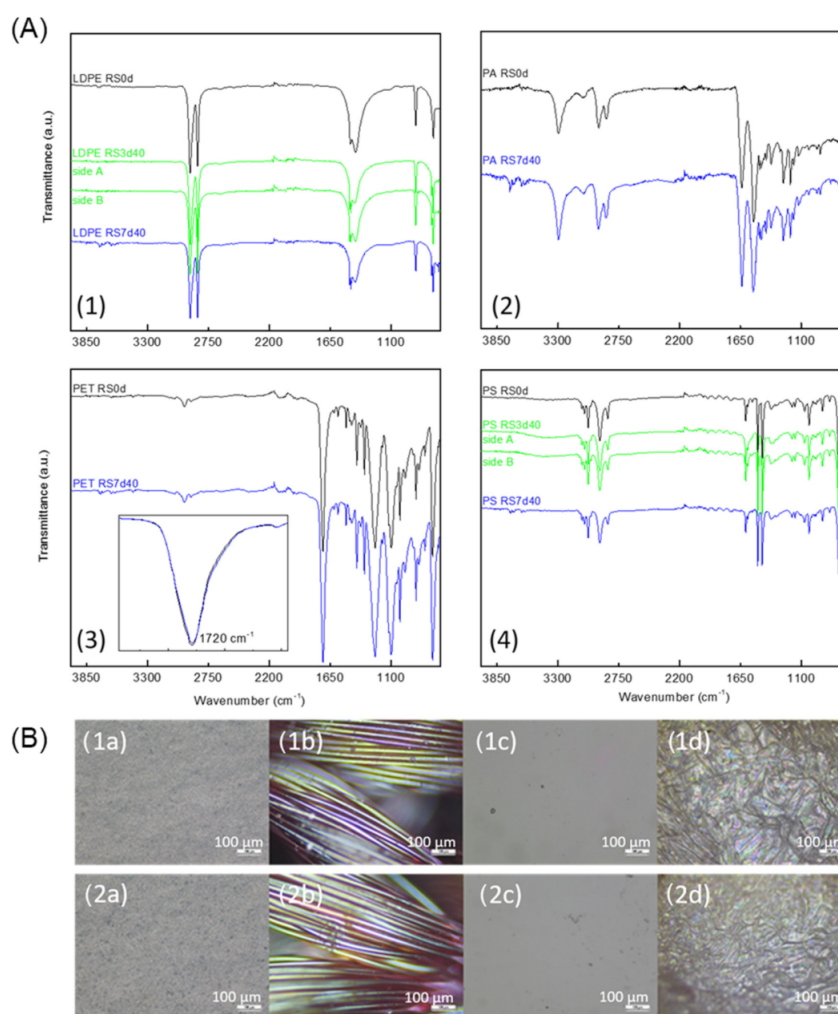
Figure 3. (**A**) FTIR spectra of RS samples of LDPE (1), PA (2), PET (3) and PS (4) at different test times in H_2O_2 at 15% (*v/v*) at 40 °C and (**B**) Optical microscopy images of RS samples (LDPE (a), PA (b), PET (c) and PS (d)), before the test (1) and after one week in H_2O_2 15% (*v/v*) (2). Samples are identified as RSXdY, where X and Y indicate the number of days and the treatment temperature, respectively.

3.2.2. Samples Tested with Enzymatic Detergent at 100% (*v/v*)

Figure 4 shows the spectra of the polymeric materials treated with the enzymatic detergent, together with the corresponding optical microscopy images. Spectra are shown in the region corresponding to the main changes detected.

The absorption spectra present modifications for LDPE and PS, suggesting potential effects on these materials by the enzymatic detergent, while PA and PET seem not to be affected. Regarding LDPE samples after one week, new absorption bands emerged around the 1000–1200 cm^{-1} region (see Figure 4A1), the spectral range of carbon-oxygen bonds from the oxidation of the material [24,25]. On the other side, after 24 h, PS spectra showed a widening of the band at 3400 cm^{-1} related to hydroxyl groups, together with the presence of peaks at 1112 and 1221 cm^{-1} related to the C-O bond stretching of esters, which could indicate an oxidation process. The spectra corresponding to PA and PET did not show important differences through the test with this solvent. The potential effects cited for LDPE and PS, on the other hand, were not evident in the surface of materials shown by optical microscopy images.
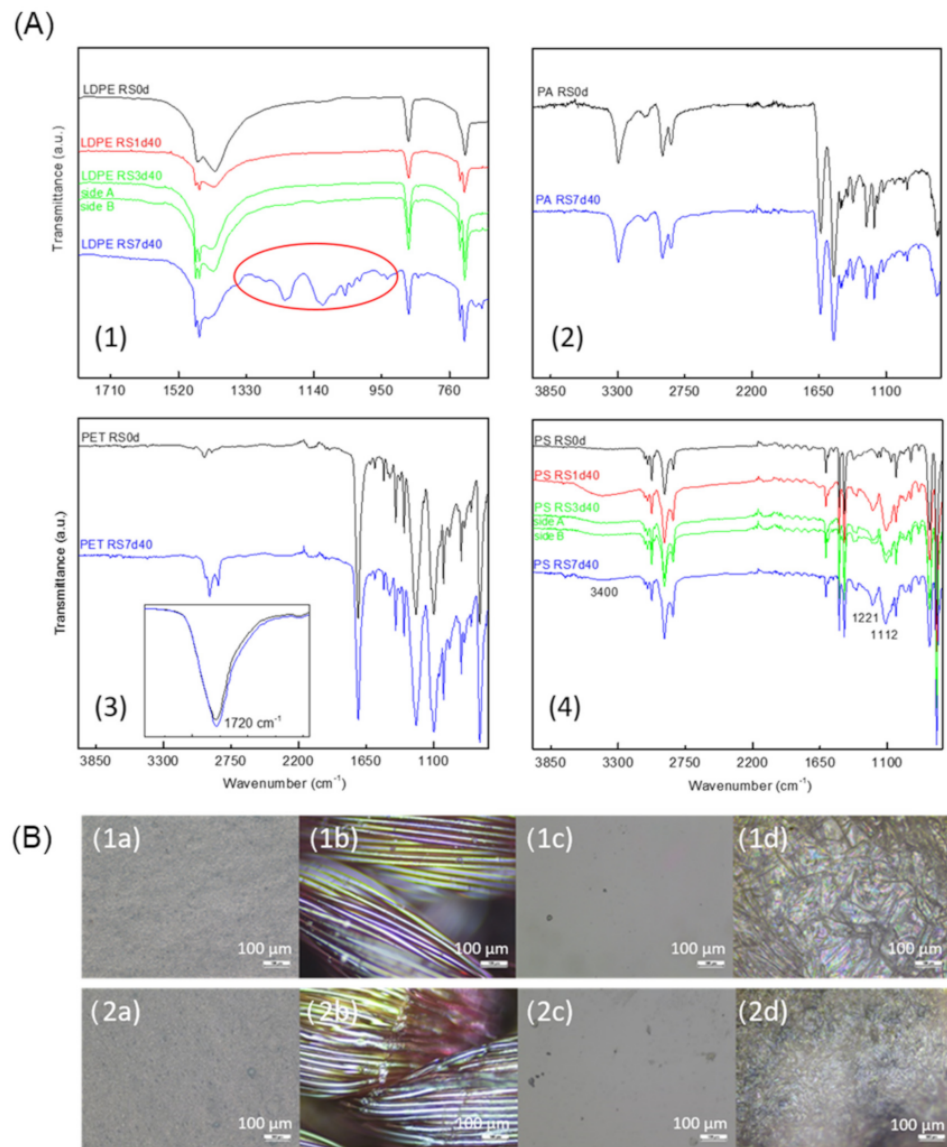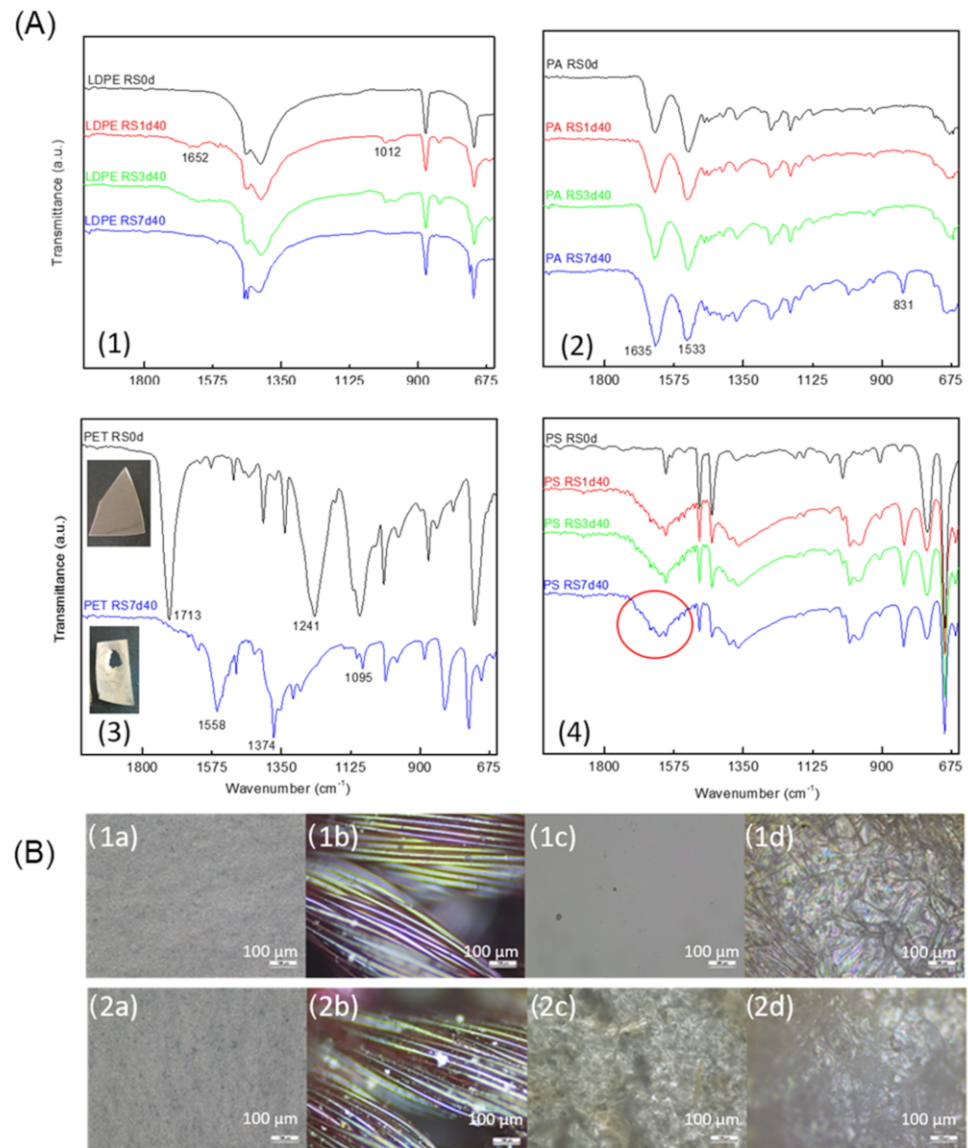
Figure 4. (**A**) FTIR spectra of RS samples of LDPE (1), PA (2), PET (3) and PS (4) at different test times in enzymatic detergent at 40 °C and (**B**) Optical microscopy images of RS samples (LDPE (a), PA (b), PET (c) and PS (d)), before the test (1) and after one week in in the enzymatic detergent (2). Samples are identified as RSXdY, where X and Y indicate the number of days and the treatment temperature, respectively, and the red circle indicates a spectral region of interest mentioned in the text.

3.2.3. Samples Tested with KOH at 20% and 10% (v/v)

The spectra related to samples tested with KOH at 20% (v/v) are shown in Figure 5.

For this solvent, changes in the spectra of all materials could be observed. In the case of PA, an intensity decrease of the band at 1533 cm^{-1}, when compared to that of 1635 cm^{-1} (corresponding to amide II and amide I regions, respectively [26]) and the presence of the band at 831 cm^{-1} related to the crystalline α form could indicate some effect on the structure. The main change of PS spectra during the test was the widening of the band at around 1600–1700 cm^{-1}. Apart from the band corresponding to the C=C stretching vibration at around 1600, the presence of carbonyl groups from the oxidation process at around 1700 cm^{-1} for treated samples could explain the broadening of this zone of the spectrum.

In the case of LDPE, the presence of two new small bands after 24 h, centered at 1652 and 1012 cm^{-1} and related to carbonyl groups [27,28] was the main difference with the spectra of untreated LDPE, possibly indicating some oxidation process of the polymer.

Figure 5. (**A**) FTIR spectra of RS samples of LDPE (1), PA (2), PET (3) and PS (4) at different test times in KOH 20% at 40 °C and (**B**) Optical microscopy images of RS samples (LDPE (a), PA (b), PET (c) and PS (d)), before the test (1) and after one week in KOH 20% (2). Samples are identified as RSXdY, where X and Y indicate the number of days and the treatment temperature, respectively, and the red circle indicates a spectral region of interest mentioned in the text.

Regarding PET samples, they seemed to be the ones most affected by the solvent. Significant changes in some absorption bands of the spectrum were evident, especially after one week (Figure 5A). The characteristic band at 1713 cm^{-1} attributed to C=O ester group [26,29,30] almost disappeared after one week, in the same way as that at 1241 cm^{-1} (corresponding to asymmetric C-O stretching vibration) [30]. The band at 1095 cm^{-1} (corresponding to symmetric C-O stretching vibration) [29] significantly decreased after one week. On the other hand, the intensity of bands at 1558 and 1374 cm^{-1} (related to symmetric and asymmetric C-H bonds, respectively) [26] increased significantly. Thus, as some bands related to ester groups decreased or tended to disappear, a change in the

structure of PET seemed to be evident. Furthermore, the modification in the structure of PET during the test seemed to be also evident by visual observation (see inner image in FTIR spectrum of PET in Figure 5A) as well as by optical microscopy (Figure 5B), where deep affections in the surface of the PET were visible, while no effects on the surface of the rest of the materials were found.

With regard to KOH at 10% at RT treatment, changes appeared to be slighter than those with KOH at 20% at 40 °C. For LDPE, PA and PET samples, the spectra (shown in Supplementary Material Figure S1) did not show differences (in agreement with Karami et al. (2017) [4], whose Raman spectra of PET remained unaltered), reflecting the effect of solvent concentration on samples. However, regarding to optical microscopy observation, small cracks were found in the surface of the PET, while no effects on the surface of the rest of the materials were evident (Supplementary Material Figure S1). This modification in the structure of PET due to KOH at 10% treatment was also visually observed as it became translucent after 72 h (Table 2). Karami et al. (2017) [4] also evidenced this effect according to scanning electron microscope (SEM) results, finding erosion voids at PET surface treated by KOH at 10% for 96 h at 40 °C, increasing this effect with temperature. The FTIR spectra of PS showed more observable modifications that could evidence a material affection after 24 h in KOH at 10%, in a similar way to that found in the case of KOH at 20%. Therefore, PS seemed to be a KOH-sensitive material even for 10% (v/v) concentration, contrary to that observed by Karami et al. (2017) [4], who did not show remarkable changes in the Raman spectra of PS after one week of treatment in KOH 10% at 40 °C.

3.3. Discussion of Main Results

Visual monitoring of organic matter and biofouling digestion in AQ and MS samples, respectively, indicated that ethanol and KOH (at both 20% and 10% concentrations) solvents were not efficient enough for the removal of these organic materials. On the other hand, H_2O_2 at 15% (v/v) and enzymatic detergent treatments at 40 °C applied during one week, revealed a good performance on organic matter and biofouling removal from AQ and MS marine collected samples. By the physical-chemical analysis based on FTIR and optical microscope for the four RS reference virgin polymers tested (LDPE, PA, PET and PS), the treatment with H_2O_2 at 15% (v/v) did not seem to affect any of the reference materials. Nonetheless, enzymatic detergent may affect the chemical structure of LDPE and PS, as revealed by the modifications identified at FTIR spectra, suggesting a potential chemical oxidation process.

Although KOH did not show good results for organic material digestion in AQ and MS samples, a further physical-chemical characterization of RS reference polymers was also carried out for treatments with KOH at 20% and 10%, as this solvent is widely used in the bibliography. However, results revealed changes in the chemical structure for all polymers tested (LDPE, PA, PET and PS), with the treatment with KOH at 20% noticeably altering the chemical structure of PET. In this case, some surface modifications were also observed for PET samples, with the material presenting small cracks on the optical microscope image, while the surface became translucent within 24 h, according to visual observations. Lower concentrations of KOH solvent also showed surface affections in PET material, even if was not reflected on the FTIR chemical characterization. Additionally, PS seemed to be chemically affected even by the lower concentration. Thus, this study highlights the unsuitability of KOH as a reagent for removing organic matter in microplastics studies or for biofouling digestion from marine-collected plastics, based on its low efficiency and the degradation caused in some polymers.

To further analyze any degradation process in the materials due to the digestion treatments, carbonyl index (CI = I_{1720}/I_{1410}) can be calculated to provide quantitative data in the case of PET, being the only material of the four evaluated with intensity bands in the carbonyl region (1720 cm^{-1}). Thus, after normalizing the spectra in the band corresponding to 1410 cm^{-1}, it can be observed (see inner image in Figure 3A3, Figure 4A3 and Figure S1A3) that comparing to the CI ratio of the initial PET material (CI = 2.852),

CI remains in very similar values after treatments in H_2O_2 at 15% (CI = 2.878), enzymatic detergent (CI = 3.024) and KOH at 10% (CI = 2.928). These results corroborate the absence of significant chemical degradation in the PET material due to these solvents. In the case of KOH at 20%, the severe modification of the chemical structure of PET evidences the disappearing of carbonyl groups.

Based on these outcomes, H_2O_2 at 15% (*v/v*) applied at 40 °C during one week has been identified as the optimum protocol for effectively reducing the organic matter and biofouling from marine-collected samples, while also preserving plastic materials for characterization. This treatment was successfully applied with no need of any additional stirring or ultrasonic methods. Nonetheless, in cases of samples containing very recalcitrant organic matter, these auxiliary steps may be taken into account for method optimization. Applying higher solvent concentrations or treatment temperatures is not recommended in these cases, as they may cause degradation of some polymers [4,16]. On the contrary, in cases of slighter organic matter or biofouling to remove, applying shorter incubation times may be considered.

4. Conclusions

Both H_2O_2 at 15% (*v/v*) and the enzymatic detergent at 40 °C are effective solutions for removing organic matter from the evaluated samples. In particular, H_2O_2 at 15% (*v/v*) is the solvent with the highest capacity to remove organic matter from the marine-collected samples, without apparently altering the physical-chemical structure of the commercial polymers tested after one week of treatment, based on their FTIR spectra, besides visual and optical microscopy observations. The enzymatic detergent is also effective for the removal of organic matter, but could affect the structures of some plastics as LDPE and PS, as detected by FTIR analysis.

Ethanol, although not affecting the structure of the tested plastics, is not effective in digesting organic matter. In contrast, the KOH at 20% (*v/v*) and 40 °C could exert a significant alteration mainly on the chemical structure of PET (probably due to its oxidation), and also affect PS at a lower degree. In the case of PS, such alteration is also evident even with KOH at 10% (*v/v*) applied at RT, indicating that this material is sensitive to this solvent even at lower concentrations and temperatures than the ones previously used.

Therefore, the solution of H_2O_2 at 15% (*v/v*), in which samples are placed for one week at 40 °C, is the most suitable for the elimination of organic matter, both from seawater samples in order to identify microplastics, as for the treatment of the surface of submerged macroplastic materials, removing the biofouling in a timely and cost-effective way and guaranteeing their integrity.

Conflicts of Interest: The authors declare no conflict of interest.

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